IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

EGGEN et al

Serial Number:	10/693,802	Group: 1639	
Filed:	October 3, 2003	Examiner:	Epperson, Jon D.

For: PROCESS FOR RAPID SOLUTION SYNTHESIS OF PEPTIDES

DECLARATION UNDER 37 C.F.R. §1.132

1. I, Ivo Franci Eggen, declare as follows:

2. I am a chemist, presently employed as Senior Project Manager Peptides at Diosynth B.V., Oss, the Netherlands.

3. I am a co-inventor of the referenced patent application, USSN 10/693,802 (hereinafter referred to as "the '802 application"), for which this declaration is submitted.

4. I am familiar with the contents of the '802 application. In addition, as a peptide chemist with over ten (10) years experience, I am familiar with the disclosures of Carpino, L.A. *et al.* (J. Org. Chem. 1999, 64, 4324-4338) (hereinafter referred to as the "Carpino article") and Solomons, T.W.G. (Organic Chemistry Fifth Edition. New York: John Wiley and Sons. 11992, page 94, Table 3.1) (hereinafter referred to as the "Solomons article").

5. Claims 28-54 of the '802 application disclose various embodiments of the DioRaSSP® (Diosynth Rapid Solution Synthesis of Peptides) method for the solution-phase synthesis of peptides. An embodiment of DioRaSSP® is explained as follows:

6. With the DioRaSSP® approach, the growing peptide is essentially anchored in a permanent organic phase (ethyl acetate in an embodiment) by means of its hydrophobic C-terminal and side-chain protecting groups. A peptide synthesis performed according to the DioRaSSP® protocol is completely homogeneous and its intermediates are not isolated. Excess reagents and by-products are intermittently removed by aqueous extractions, and no organic waste streams are generated during performance of the synthesis. The DioRaSSP® protocol is a repetitive method involving a number of synthetic cycles. As shown in Figure 1, one cycle of an embodiment of the DioRaSSP® protocol consists of a coupling step, scavenging of residual activated carboxylic compound, aqueous extractive work-up, deprotection of the N-terminal amino function, and finally another aqueous extractive work-up. Couplings are mediated by water-soluble carbodiimide to allow aqueous extractive work-up and avoid an additional filtration step.

7. After completion of a coupling, residual activated carboxylic compound, if hydrophobic, is scavenged with a compound containing a nucleophilic moiety (*e.g.*, an amine), which is able to convert an activated carboxylic moiety, as well as an anion-forming moiety, which can be deprotonated under mildly basic aqueous conditions compatible with peptide synthesis (*e.g.*, a protected or free carboxylate, sulphonate or phosphonate). An embodiment of a scavenging compound is a β-alaninate ester, the lability of which is

similar to that of the temporary amino-protecting function, allowing simultaneous deprotection of the growing peptide and the scavenged compound. The DioRaSSP® approach assures the completely quantitative removal of scavenged compounds before the coupling step of the next cycle of the synthesis by basic aqueous (that is: active) extraction, while the growing peptide remains anchored in the organic phase due to the presence of hydrophobic protecting functions.

8. Several repetitive methods for peptide synthesis in solution have been reported in literature. The most recent example is the method developed by Carpino, L.A. and co-workers and is disclosed in the Carpino article. The Carpino article reported the use of a(n) (poly)amine as a scavenger for residual activated carboxylic compounds in addition to the application of the group 1,1-dioxobenzo[*b*]thiophene-2-ylmethoxycarbonyl (Bsmoc) as amino-protecting in the process. The Bsmoc function has very high lability towards base. As a result, in one step residual activated carboxylic functions are scavenged and Bsmoc functions are removed using a(n) (poly)amine. Thus, in the Carpino process, deprotection of the N-terminal function necessarily takes place under the same reaction conditions as the scavenging of excess activated carboxylic functions.

9. The Carpino article further discloses experiments performed to gain a general picture of the ease of the deblocking process, using a number of simple deblocking amines. The following reactivity order was observed: piperidine > piperazine > morpholine ≈ ethanolamine. The lowest reactivity rate was observed with the primary amine ethanolamine.

10. Ethanolamine in water is present in the non-dissociated form $NH_2CH_2CH_2OH$, the pK_a of water being lower (15.74) than that of ethanolamine (16) (see the Solomons article). In water the alcohol function of ethanolamine cannot be deprotonated, as the hydrogen in the hydroxy group of ethanolamine is less acidic than the hydrogens of the water molecules. The alcohol function of ethanolamine can only be deprotonated using a very strong base, such as potassium hydride, in non-aqueous solvents. Accordingly, such conditions are incompatible with peptide synthesis involving aqueous conditions.

11. As a chemist and co-inventor of this case, and as a person skilled in the art, I declare, that the various scavengers used in claims 28-54 of the '802 application are different from the scavengers used and disclosed by the Carpino article. Scavengers disclosed in claims 28-54 of the '802 application comprise an anion-forming moiety, which can be deprotonated under mildly basic aqueous conditions compatible with peptide synthesis, whereas the scavengers used and disclosed by the Carpino article do not comprise such a moiety.

12. In the solution synthesis of peptides according to the Carpino article, the alcohol functionality of ethanolamine remains intact during aqueous washings and does not form an anion.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like

so made are punishable by fine or imprisonment, or both, under 17 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

20-01-2006
Date

I.F. Eggen

Fig. 1. A synthesis cycle according to Z-DiosRaSSP.

(Here, the benzyloxycarbonyl (Z) function is applied for temporary amino protection.

This function is removed by hydrogenolysis in each cycle of the process.)